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GAMETOPHYTES OF FIVE SPECIES OF RAJASTHAN FERNS UNDER CULTURAL CONDITIONS

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ABSTRACT

Gametophytes of seven species of ferns raised from spores on agar medium, are described. These are either cordate (*Cheilanthes, Hypodematium, Cyclosorus* and *Tectaria*) or lobed, thalloid (*Actiniopteris, Asplenium* and *Adiantum*). All are homosporous ferns but in some of the taxa distinction is observed between antheridia and archegonia bearing prothalli (*Cheilanthes, Hypodematium* and *Tectaria*). Variations in morphology, sex expression and sporophyte formation are discussed.

INTRODUCTION

In Rajasthan approximately 40 species of ferns belonging to 19 genera are found, distributed in the Aravalli Hills and the Chambal ravines (Bohra *et al.*, 1980, Bhardwaja *et al.*, 1987, Sharma *et al.*, 1988). Majority are homosporous, and produce sporangia in sori. The spores show wide variations in morphology and size (Sharma and Sharma, 1988). On germination the spores produce gametophytes which are of different shapes, sizes and in sex expression. In the present paper gametophytes of seven species of common ferns of Rajasthan are described.

MATERIALS AND METHODS

Spores collected from mature sori are stored in the 'spore bank' and properly labelled. The spores were sterilized prior to germination in 3% Sodium hypochlorite for two minutes (Faiure, 1975) and sown on agar medium supplemented with Knop's solution (half-strength) in sterilized conical flasks. The cultures were kept in a Chamber with continuous light, approximately 75% relative humidity and temperature ± 20°C. Observations were recorded every 10th day till the formation of sporophytes *i.e.*, for nearly 4 months. Gametophytes were transferred to fresh medium as and when required.

DESCRIPTION

The ferns selected for the present study are *Actiniopteris radiata, Adiantum philippense* (*A. lunulatum*), *Asplenium pumilum* var. *hymenophylloides, Cheilanthes farinosa, Cyclosorus dentatus, Hypodematium crenatum* and *Tectaria macrodonta.* Table 1 shows the time taken by various stages of development of gametophytes, sex expression and sporophyte formation. Morphology of mature gametophytes of the above mentioned taxa is given below :

Actiniopteris radiata (Sw.) Link : The mature gametophyte is an irregularly-lobed, green thalloid structure (Plate 1A, Fig. 1A).

The cells of the wing are hexagonal and each possesses numerous chloroplasts. The ridge is seen only in basal portion and

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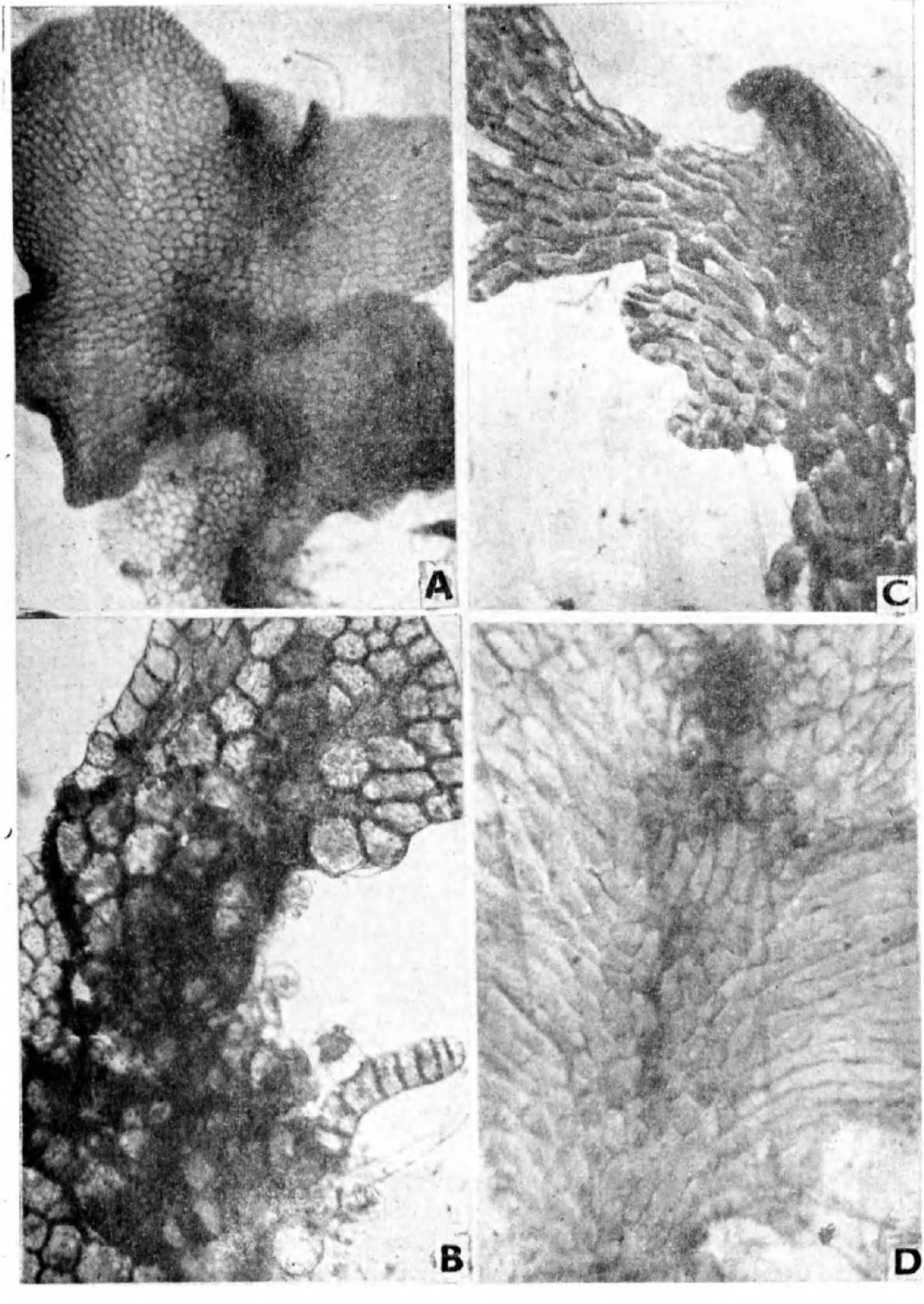

Plate. 1: (A-D): A. *Actiniopteris radiata*, lobed thalloid gametophyte × 18. B. Same, antheridia on filamentous outgrowths × 36. C. *Adiantum philippense*, irregular, lobed gametophyte made up of linear cells and a young apogamous sporophyte × 18. D. same, gametophyte with diverging lateral cells and a vascular strand × 18.

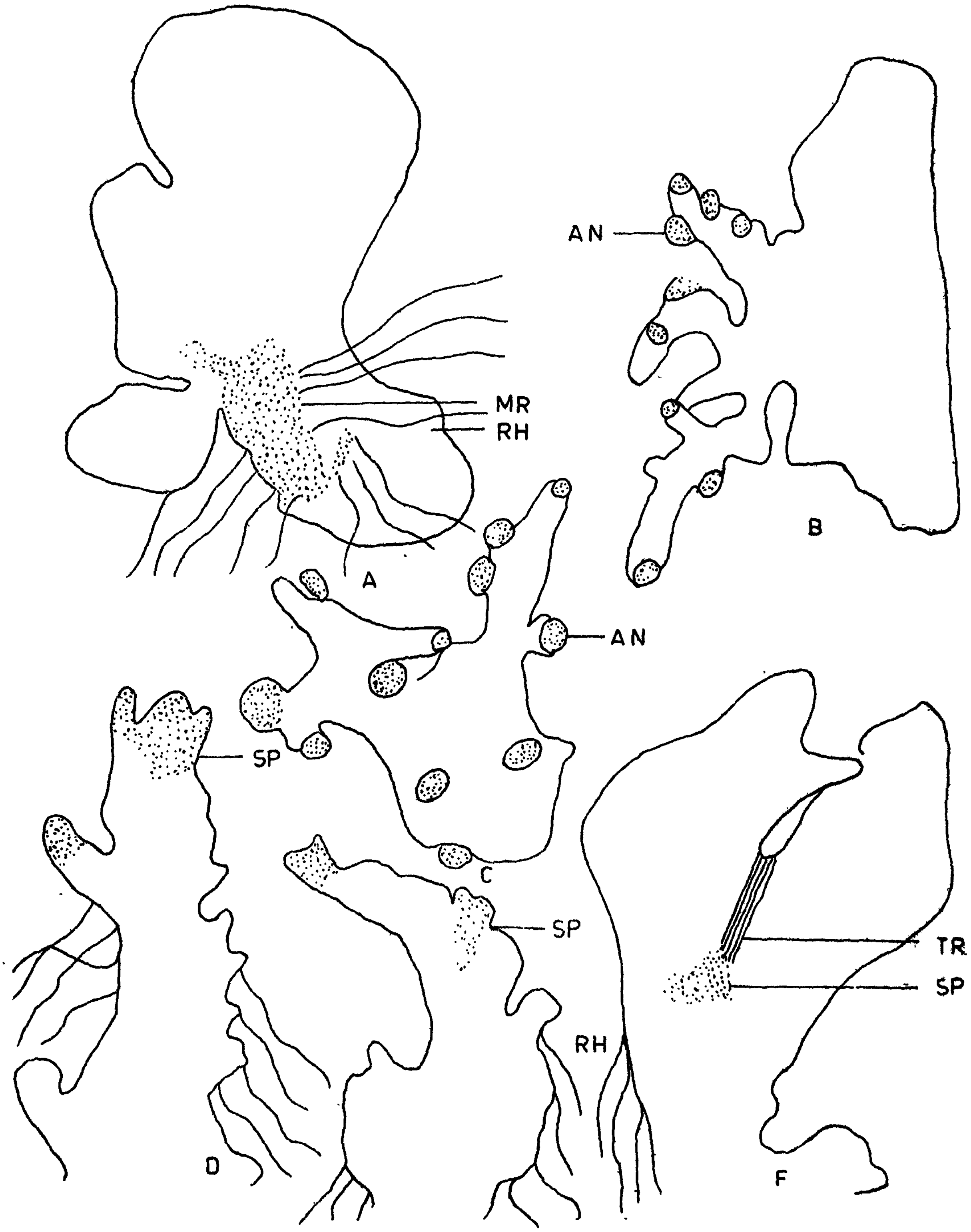

Fig. 1 : (A-F): A. *Actiniopteris radiata,* irregular lobed gametophyte with a small ridge in basal portion × 18. B,C. Same, irregular prothalli with antheridia on filamentous out-growths × 24. D-F. *Adiantum philippense,* irregular or cordate non-ridged gametophytes with sporophytes, originating from. different portions of the prothalli. Vascular strand is also seen in F. × 18 (MR—median ridge, RH—rhizoid, AN—Antheridium, SP—Sporophyte, TR—Vascular strand).

numerous rhizoids arise from it. The rhizoids are large, smooth-walled, multicellular structures. No sex organs could be seen in 60-70 day-old gametophytes. However, in 75-80 day-old prothalli, lateral filaments and irregular projections originate (Plate 1B, Fig. 1B, C) which bear antheridia. The antheridia are terminal as well as lateral structures. Each consists of a three-celled wall and a number of multiflagellate antherozoids.

Archegonia are not produced usually, however, in gametophytes treated with 10 ppm IAA, archegonia do develop (Sharma 1989, Sharma 1990) on the lateral branches produced from the basal ridge portion of the gametophyte. Apogamous sporophytes are produced frequently from the anterior portion of the ridge.

When cut pieces of roots of apogamous sporophytes are cultured on agar medium supplemented with Knop's solution (half strength), a number of green thalloid prothalli are produced. These are also devoid of sex organs. After 50-60 days, a number of apogamous sporophytes are produced which are identical to the parent plant of *Actiniopteris radiata*. But sori did not develop on these sporophytes.

Adiantum philippence L. (*A. lunulatum*) : The gametophytes are noncordate, linear, lobed, thalloid structures (Plate 1 C, D; Fig. 1 D-F, 2 A-D).

The median ridge is not seen. The cells of the wing are distinct, linear to squarish and comparatively larger in size than those of other gametophytes described in the present paper. Strands of linear tracheids-like cells are visible in several gametophytes (Plate D, Fig. 1F) Mehra and Gupta (1986) have also shown similar cells in *A. lunulatum*. Antheridia bearing gametophytes (Fig. 2D) are comparatively smaller and produce large number of globular, protruding antheridia. The antheridial wall is three-celled structure and a number of antherozoids are produced from each. Archegonia could not be observed in any gametophyte studied for the present purpose. However, sporophytic plants are seen originating in almost all prothalli after 70-75 days of sowing of spores. These originate from different portions of the gametophytes (Figs. 1D, E, 2 A-C) and adopt various shapes. In older ones scales originate from their apical portions and rhizoids from the basal region. The sporophytes produced are apogamous, as these develop from the vegetative cells of the prothallus. Their cells are different and distinct from those of the gametophyte. Tracheary elements are visible in many of these sporophytes (Plate 1C).

Hypodematium crenatum (Forssk.) Kuhn : Similar to *Cheilanthes farinosa* and *Tectaria macrodonta* two kinds of prothalli are produced *i.e.* linear, non-cordate antheridia bearing (Plate 2C, Fig. 3F) and the cordate ones with distinct median ridge and archegonia (Plate 2A, Fig. 3E).

In the latter type of gametophytes, stalked, capitate glands are present on the hexagonal cells (Plate 2B). Each gland has a distinct stalk and a globular terminal body. From the margin of cordate prothallus arises small 2-3-celled outgrowths (trichomes), each terminates into a gland. Similar trichomes occur in the gametophytes of a number of ferns of the family *Dryopteridaceae e.g. Polystichum, Acrophorus, Dryopteris* (Mehra and Gupta, 1986).

The sporophytes arise from cordate prothalli after 80 days of sowing of spores. Whether the sporophytes are produced apogamously or are sexually produced, not known.

Asplenium pumilum var. **hymenophylloides** (Fée) C. B. Clarke : The mature prothalli are non-cordate, non-ridged, irregularly lobed, thalloid structures. The cells are hexagonal to rectangular. Antheridia are produced in large numbers (Figs. 3A, B). Each is a globular outgrowth with three-celled wall. A number of multi-flagellate antherozoids arise from each antheridium. Archegonia bearing gametophytes could not be observed. Similarly, the presence of sporophytes was not noticed in any prothallus even after 80 days of sowing of spores.

Cyclosorus dentatus (Forssk.) Ching : The mature prothallus is a typical heart-shaped, ridged, bisexual gametophyte.

Archegonia are produced in the anterior portion of the ridge, while antheridia are seen intermingled with rhizoids. Sporophytes are usually not produced even in 70 day-old gametophytes. When these gametophytes are transferred to fresh medium supplemented with Knop's solution (half-strength), a new generation of cordate prothalli are produced from the marginal cells of the parent gametophyte (Plate 2D ; Figs. 3C, D). These second generation of prothalli possess distinct archegonia, but antheridia do not appear. After 20-30 days of re-transfer to a fresh medium, a third generation of cordate, ridged gametophytes is produced from the marginal cells of the secondary prothalli (Fig. 3C). These tertiary gametophytes also possess archegonia similar to that of earlier generations, but no antheridia. Sporophytic plants originate from the secondary and tertiary gametophytes. Thus, from a single spore, number of sporophytic plants are produced. The authors could transfer satisfactorily the sporophytic plants thus raised in culture-flasks to the earthen pots in the garden of the Department. The sporophytic plants are similar in morphology to typical plants of *Cyclosorus dentatus* but sori did not appear on these plants ; reason is unknown.

Cheilanthes farinosa (Forssk.) Kaulf : Two kinds of prothalli are produced : small, linear, non-ridged antheridia-bearing (Plate 3B) and large, cordate, ridged prothalli with distinct archegonia (Pl. 3A).

Antheridia are globular, protruding bodies, each with three-celled wall and liberates large number of multi-flagellate antherozoids. Archegonia have distinct, protruding neck, which may be straight or curved. Sporophytic plants are raised frequently from the ridge portion of the prothalli.

Tectaria coadunata (J. Sm.) C. Chr. *Tectaria macrodonta* (C. Chr.) : Similar to *Hypodematium crenatum* and *Cheilanthes farinosa*, two kinds of prothalli are produced in *T coadunata*. The linear, non-cordate prothalli are comparatively smaller in size and bear antheridia on abaxial surface (Plate 3D ; Fig. 2E). The cordate, ridged, archegonia-bearing prothalli (Plate 3C ; Fig. 2E) are large and have smooth or wavy margins. Archegonia are produced on the entire length of the ridge. The sporophytic plants arise frequently from these gametophytes. Whether these are produced sexually or are apogamous, is not yet studied.

DISCUSSION

Excluding Marsileales and Salviniales, all other lepto-sporangiate ferns are described as homosporous ferns (Nayar 1970, Nayar and Kaur 1974). However, morphological variations have been reported among the spores collected from a single sporophyll in some of the taxa of homosporous ferns, for example in *Cheilanthes albomarginata*, the spores may be circular or elliptical with trilete or monolete laesura (Sharma and Sharma 1988). Similarly, different sizes of spores are also known to exist in some of the taxa of homosporous ferns (Santha Devi 1977). The occurrence of two kinds of prothalli *i.e.*, antheridia bearing and archegonia-bearing in some of the taxa of ferns *e.g. Cheilanthes farinosa* (Sharma and Vangani 1988), *Hypodematium crenatum* and *Tactaria coadunata*

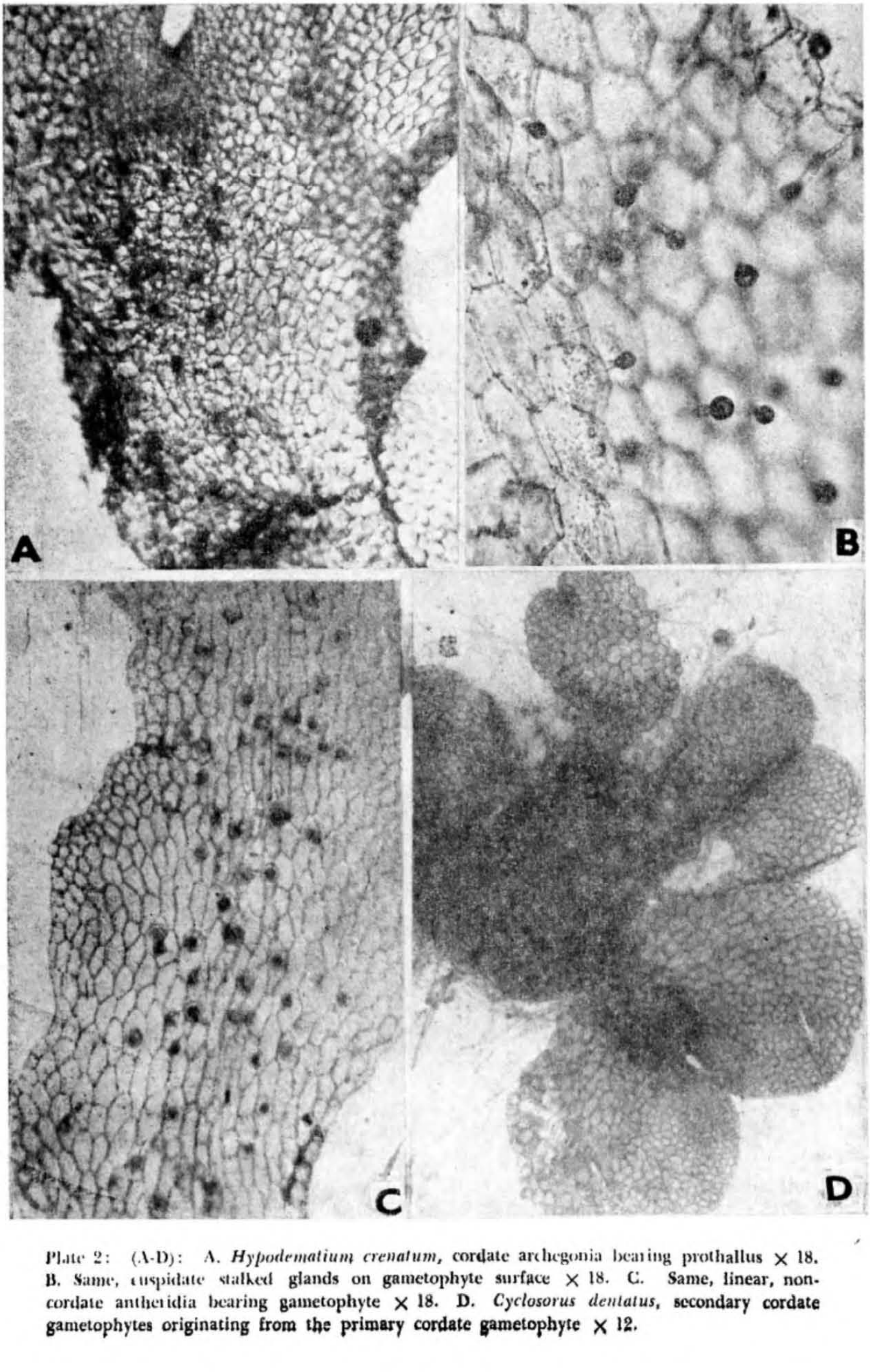

Plate 2: (A-D): A. *Hypodematium crenatum*, cordate archegonia bearing prothallus × 18. B. Same, cuspidate stalked glands on gametophyte surface × 18. C. Same, linear, non-cordate antheridia bearing gametophyte × 18. D. *Cyclosorus dentatus*, secondary cordate gametophytes originating from the primary cordate gametophyte × 12.

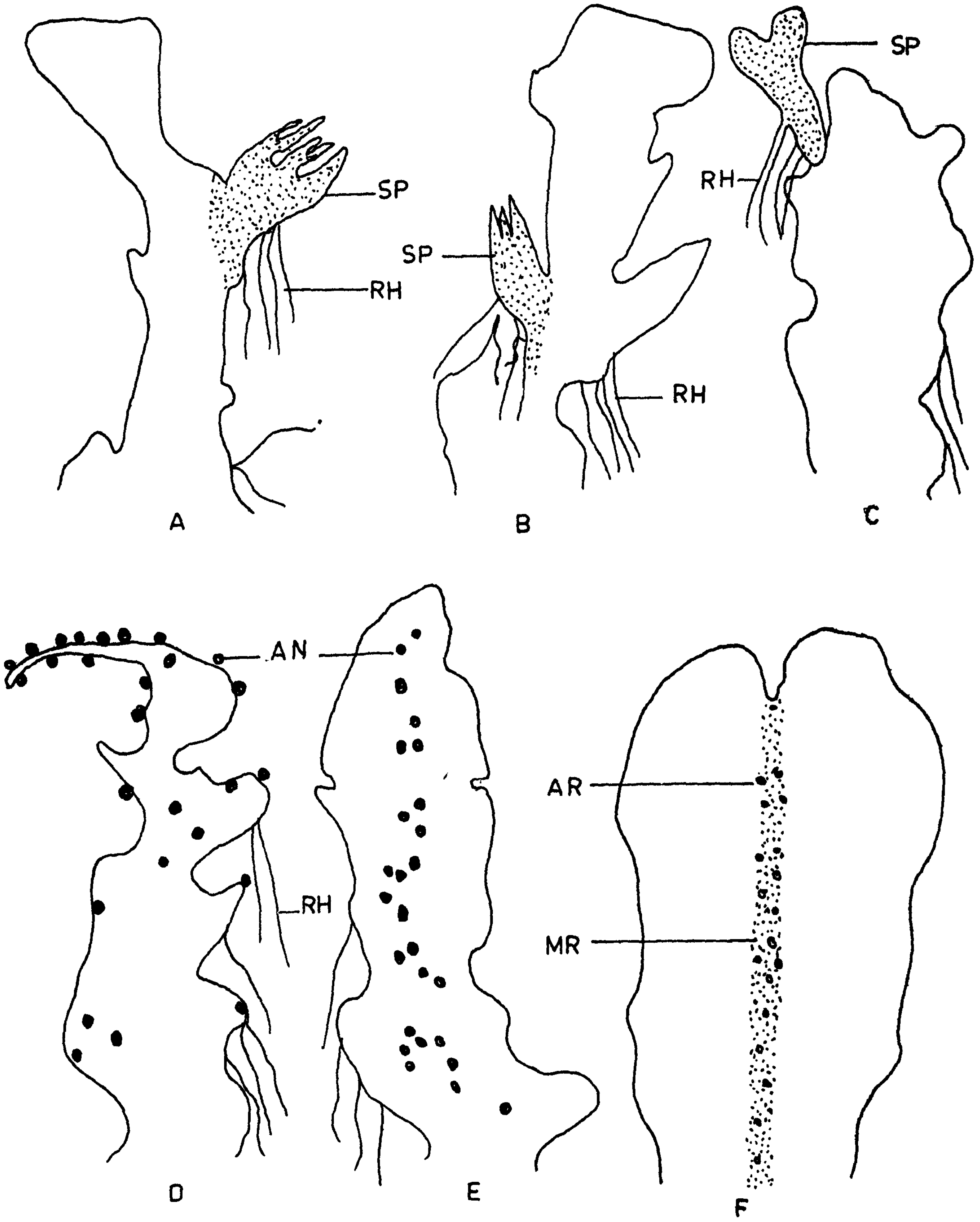

Fig. 2: (A-F): A-C. *Adiantum philippense* irregular lobed, non cordate gametophytes with sporophytes of various sizes × 18. D. Same, an antheridia bearing gametophyte × 18. E. *Tectaria coadunata*, linear, non-ridged male gametophyte bearing antheridia × 18. F. Same, a cordate, ridged archegonia bearing gametophyte × 18 (SP—sporophyte, AN—Antheridium, RH—Rhizoid, AR—Archegonium, MR—Median ridge).

described above, suggests the existence of incipient heterospory identical to the one proposed in the genus *Equisetum* (Mohan Ram and Chatterji 1970). Rather, in ferns, it is more advanced than in *Equisetum* as the two kinds of gametophytes are quite distinct in morphology, whereas, in *Equisetum*, all male, female and bisexual prothalli are identical and only in sex expression they show differences.

The present investigation also throws light on methods of multiplication of ferns through apogamy and apospory. Formation of sporophytic plants in *Actiniopteris* are apogamous as sex organs are usually absent in this plant (Stokey 1948, Nayar 1962). Formation of large number of gametophytes from the surface cells of the cut pieces of roots sown on agar medium and supplemented with Knop's solution (half-strength) are aposporous. These

Table 1: Spore germination, prothallus development, sex expression and sporophyte formation in some ferns of Rajasthan

Days	10—15	20—25	30—35	40—50	50—55	60—65	70—75	80—85
Plants								
Actiniopteris radiata	Spore germination	Plate formation	—	—	Lobed prothallus	Young sporophytes	Antheridia	Sporophytes
Adiantum philippense	—	Spore germination	Plate formation	—	Mature proth.	,,	,,	,,
Hypodematium crenatum	Spore germination	Plate formation	—	Prothalli 2 types	Linear with Antheridia	—	Cordate with Archegonia	Few sporophytes
Asplenium pumilum	,,	,,	—	Mature proth.	Anth.	—	—	—
Cyclosorus dentatus	,,	,,	—	,,	Anth. & Arch.	—	Sec. & Tertiary Prothalli on transfer	Sporophytes
Cheilanthes farinosa	,,	,,	—	Prothalli 2 types	Anth. on linear	Arch. on cordate	—	Sporophyte
Tectaria coadunata	,,	,,	—		Anth. on linear Arch. on cordate	—	—	Sporophytes rare

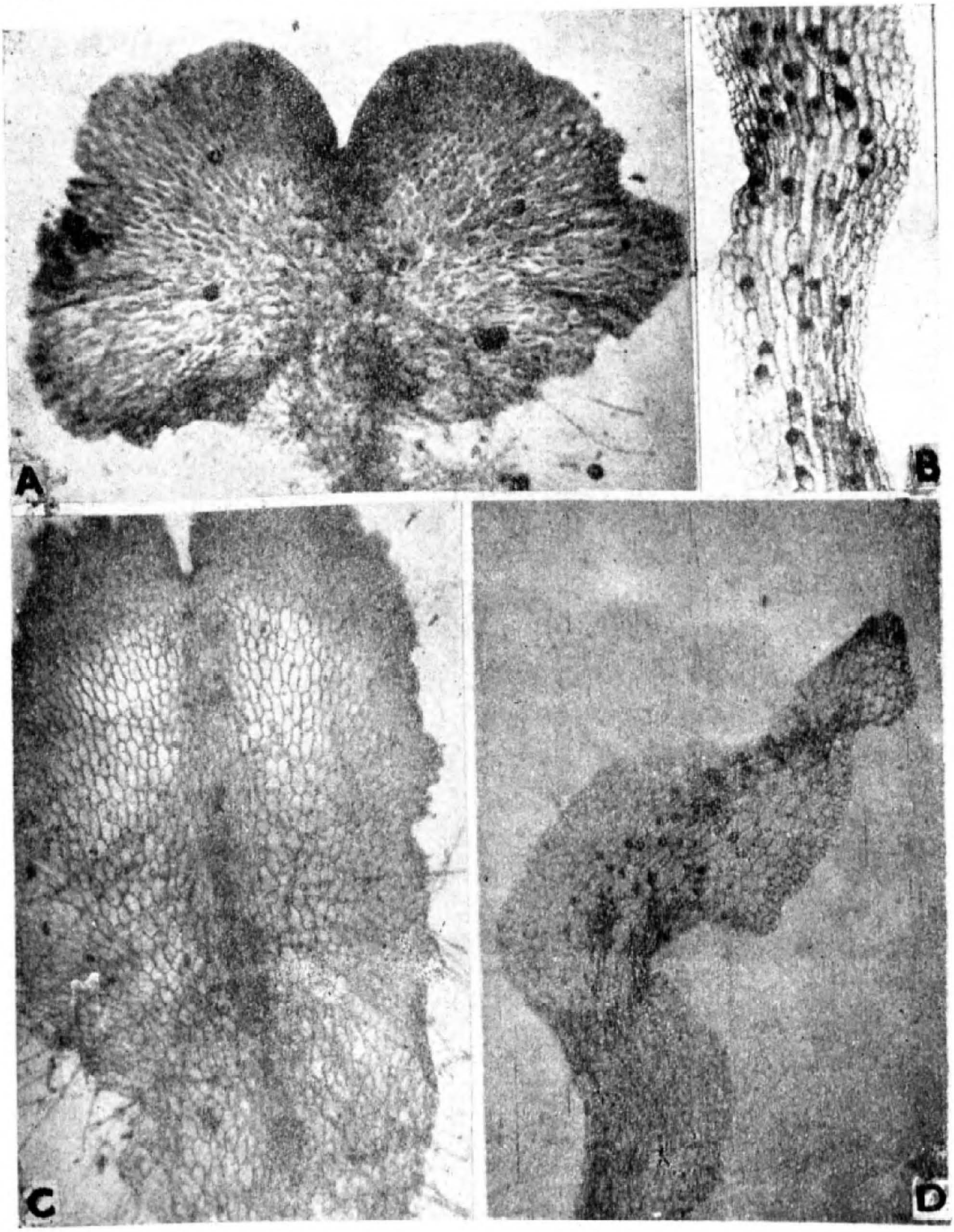

Plate 3. (A-D): A. *Cheilanthes farinosa*, cordate, archegonia bearing gametophyte × 18. B. Same, a linear antheridia bearing gametophyte × 12. C. *Tectaria coadunata*, cordate ridged archegonia bearing gametophyte × 18. D. Same, antheridia bearing linear, non-ridged gametophyte × 18.

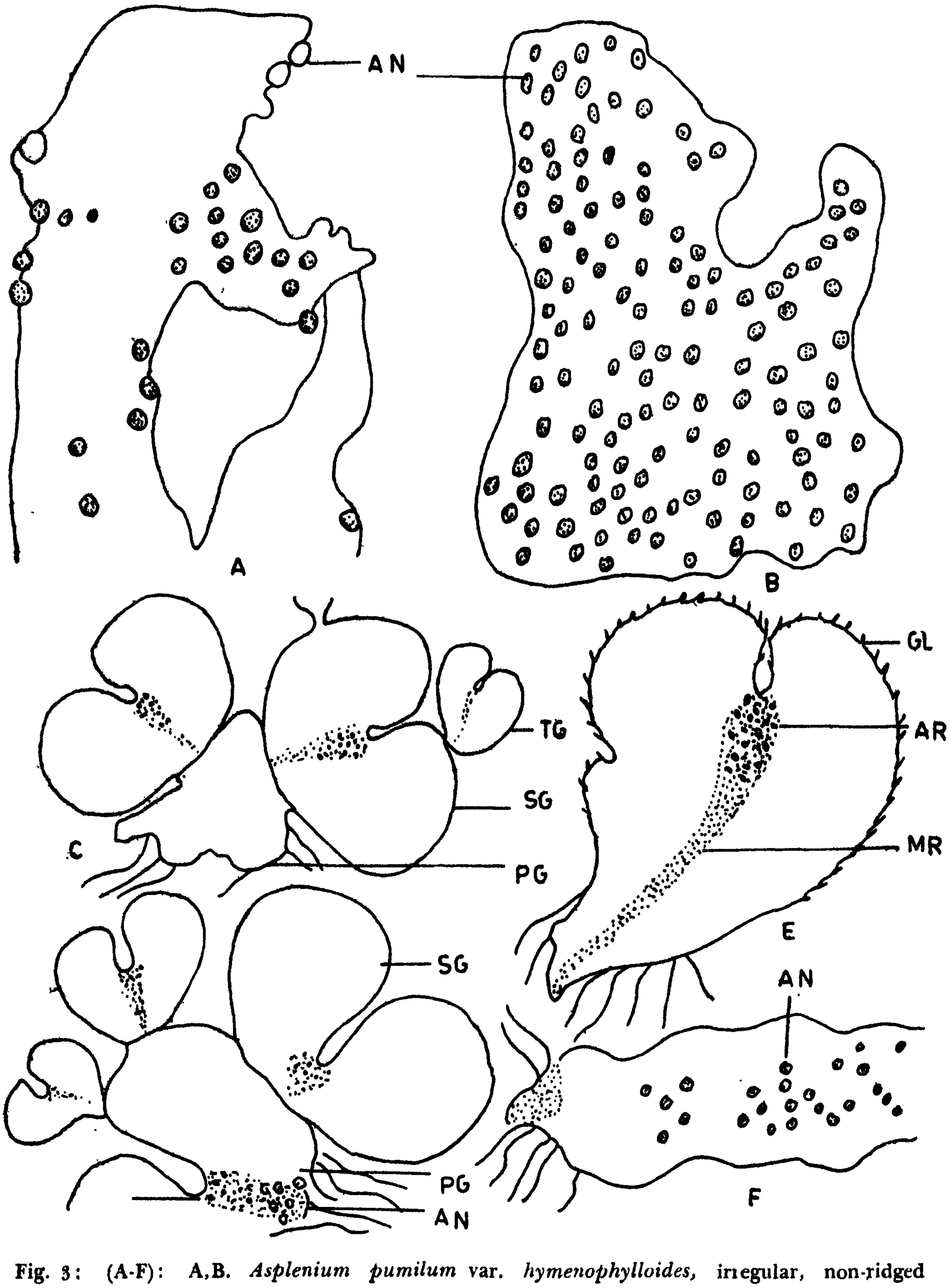

Fig. 3: (A-F): A,B. *Asplenium pumilum* var. *hymenophylloides,* irregular, non-ridged gametophytes with numerous antheridia × 18. C,D. Cyclosorus dentatus, Secondary and Tertiary cordate, ridged gametophytes seen originating from the margins of primary cordate, ridged prothalli × 12. E. *Hypodematium crenatum* cordate, ridged gametophyte with archegonia. From the margin arises a number of 2-3 celled trichomes × 18. F. Same, linear non-ridged antheridia bearing prothallus. 18 (AN—antheridia, PG—primary gametophyte, SG—secondary gametophyte, TG—tertiary gametophyte, GL—caspidate gland, AR—archegonium, MR—median ridge).

gametophytes are devoid of sex organs but apogamous sporophytes develop frequently from them. Similarly, in *Cyclosorus* origin of new generation of gametophytes from the marginal cells of the parent gametophyte, when transferred to fresh medium, are aposporous. A third generation of prothalli may also be produced aposporously.

Apogamous sporophytes are also produced in *Adiantum philippense* (*A. lunulatum*), but in young stages these are different from the typical sporophytic plant of *A. philippense* *i.e.* organogenesis is delayed; specially the roots and leaves. However, vascular strand is differentiated quite early during development.

In *Asplenium pumilum* var. *hymenophylloides*, antheridia are produced in such a large number that the material may be used for the separation of the chemical antheridogen, as was discovered in *Anemia*. Neither archegonia nor sporophytic plants developed in the gametophytes of *A. pumilum* var. *hymenophylloides.*

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